BEHAVIOR OF *LISTERIA MONOCYTOGENES* INOCULATED ON WHOLE CANTALOUPE AND FRESH-CUT PIECES

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ABSTRACT

Attachment and survival of *Listeria monocytogenes* on external surfaces (rind) of inoculated cantaloupe, transfer of the pathogen from unsanitized and sanitized rinds to fresh-cut tissues during cutting and growth on fresh-cut pieces were investigated. Surface treatment with 70% ethanol to reduce the native microflora on treated melon, followed by immersion in a four strain cocktail of *L. monocytogenes* (10^8 CFU/ml) for 10 min, deposited 4.2 $\log_{10}$ CFU/cm^2 and 3.5 $\log_{10}$ CFU/cm^2 on treated and untreated cantaloupe rinds, respectively. *L. monocytogenes* survived on the treated or untreated cantaloupe rinds for up to 15 days during storage at 4°C and 20°C, but populations declined by approximately 1 to 2 $\log_{10}$ CFU/cm^2. Fresh-cut pieces prepared from inoculated whole cantaloupes stored at 4°C for 24 h post inoculation were positive for *L. monocytogenes*. Washing inoculated whole cantaloupes in solutions containing 1000 ppm chlorine or 5% hydrogen peroxide for 2 min at 1 to 15 days storage at 4°C post inoculation resulted in a 2.0 to 3.5 log reduction in *L. monocytogenes* on the melon surface. Fresh-cut pieces prepared from the sanitized melons were negative for *L. monocytogenes*. After direct inoculation onto fresh-cut pieces prepared from sanitized cantaloupe, *L. monocytogenes* grew, but survived up to day 6 on fresh-cut pieces prepared from unsanitized cantaloupe during 15 days of storage at 4°C. Growth was enhanced in fresh-cut pieces with low population of native microflora during storage at 4°C. At 8 and 20°C of storage growth of *L. monocytogenes* on fresh-cut pieces was suppressed while populations of native microflora increased. It is concluded that sanitizing with chlorine or hydrogen peroxide has the potential to reduce or eliminate the transfer of *L. monocytogenes* on melon surfaces to fresh-cut pieces during cutting. Also the data suggests that the native microflora of cantaloupe inhibits growth of the pathogen in fresh-cut pieces.

INTRODUCTION

Listeria monocytogenes is a particular food safety concern because it is widespread in the environment (*4,10*), grows under refrigerated conditions, and is a frequent resident in certain food processing establishments (*8, 15*). The ability of *L. monocytogenes* to attach to a variety of surfaces (*23*) and its virulence characteristics also contribute to this concern. *L. monocytogenes* has been implicated as a causative agent of several foodborne outbreaks, which have resulted in both human illness and death (*16,25*). The microorganism has been isolated from soil, sewage sludge, vegetation, and water (*11,12*) and therefore, has the potential to contaminate cantaloupe surfaces.

Many vegetables, including bean sprouts, cabbage, cucumber, potatoes, and radishes have been found to be contaminated with *L. monocytogenes* (*2, 4,11, 12,17,18*). Wong et al. (*35*) detected *L. monocytogenes* in 12.2% of vegetable samples examined. An outbreak attributed to

the consumption of contaminated coleslaw (*28*) and a multistate seed sprout recall due to potential contamination with *L. monocytogenes* have occurred. Even though there are no documented reports of an outbreak of human listeriosis associated with the consumption of contaminated fresh-cut cantaloupe, the potential for such an outbreak remains a concern. Sporadic illnesses have been linked with *L. ivanovii* (*28*) and one case of meningitis with *L. seeliger* (*25*), but *L. monocytogenes* remains the major pathogen to man. In this study, we investigated the attachment and survival of *L. monocytogenes* inoculated on the surfaces of untreated cantaloupe or cantaloupe treated with 70% ethanol (EtOH) and 1000 ppm chlorine to reduce the native background microflora. Also, the efficacy of the sanitizer treatments (chlorine and hydrogen peroxide) in reducing transfer of the pathogen from the cantaloupe rinds to the fresh-cut pieces during cutting and the ability of *L. monocytogenes* to grow on inoculated fresh-cut pieces when stored at 5 and 10°C were also investigated.

MATERIALS AND METHODS

Bacterial Strains and Cantaloupe Used

Strains of *Listeria monocytogenes* used to prepare inoculum cocktails were Scott A (clinical isolate), ATCC 15313 (type strain), H7778 and CCR1-L-G (food isolate). Unwaxed cantaloupe (1721 ± 48 g) melons were purchased from a local wholesale distribution center. Melons were stored at 4°C. Before use, melons were unpacked and placed on the laboratory bench for ~18 h to allow them to come to room temperature (~20°C).

Preparation of Sanitizing Solutions

Clorox, a commercial bleach containing 5.25% sodium hypochlorite (NaOCl, Clorox Company, Oakland CA), was diluted with sterile water to provide a concentration of 1000 ppm of available chlorine in the wash solution. The pH was adjusted to 6.4 ± 0.1 by adding citric acid. Free chlorine in the solution was then determined with a chlorine test kit (Hach Co., Ames, IA) that has been approved by the U.S. Environmental Protection Agency. A 70% ethyl alcohol (EtOH) solution was prepared from a 200 proof solution (U.S.P., Warner-Graham Co., Cockeysville, MD).

Sanitizing Treatments

Before inoculating with *Listeria monocytogenes*, surfaces of whole cantaloupe were washed with water or 70 % EtOH to reduce total microflora on the surface of cantaloupe. All washing treatments were performed by submerging the melons under the surface of 3 L sterile tap water or 70% EtOH then manually rotating the melons to assure complete coverage and contact of surfaces with the wash solution for 5 min. Washed melons were placed on crystallizing dishes inside a biosafety cabinet to dry for 1 h.

Preparation of Bacterial Inoculum

A mixed bacterial cocktail containing 4 strains of *L. monocytogenes* [Scott A; ATCC 15313; H7778; CCR1-L-G] from the USDA-ARS-ERRC culture collection was cultured by two successive loop transfers at 18 h intervals (37°C) in 5 ml Trypticase Soy Broth (TSB, Difco, Detroit, MI) supplemented with 0.6% yeast extract (TSBY, Difco). A final transfer of 0.2 ml

was made into 20 ml TSBY with incubation at 36°C for 18 h under static conditions. The bacterial cells were harvested by centrifugation (10,000 *g*, 5 min) at 4°C. The cell pellets were washed twice in salt-peptone [0.85% NaCl, 0.05 % Bacto-peptone (Difco)] and the cell pellets were transferred to 3 L of 0.1 % peptone-water (inoculum cocktail, 8.58 $\log_{10}$ CFU/ml). When required the inoculum was diluted with 0.1 % peptone-water.

Inoculation of Whole Melon and Fresh-Cut Pieces

Surface sanitized and unsanitized whole melon was inoculated with *Listeria monocytogenes* by submerging the melons in 3 L of 0.1 % peptone-water containing the bacteria cocktail (8.58 $\log_{10}$ CFU/ml). In a separate experiment to determine influence of inoculum density on survival, fresh-cut pieces prepared from whole melon sanitized with 1000 ppm chlorine were inoculated with 10^3 to 10^6 CFU/ml.

Washing Treatments

Three wash treatments were compared: sterile tap water, 1000 ppm chlorine and 5% hydrogen peroxide. The 1000 ppm chlorine solution was prepared by diluting Clorox® commercial bleach containing 5.25% NaOCl in sterile deionized water and adjusting the pH to 6.4 ± 0.1 by adding citric acid (Mallinckrodt, Paris, KY). Free chlorine in the solution was determined with a chlorine test kit (Hach Co., Ames, IA) that has been approved by the U.S. Environmental Protection Agency. A 5% hydrogen peroxide solution was prepared from a 30% stock solution (Fisher Scientific, Suwanee, GA) by dilution with sterile tap water. All washing treatments were performed by submerging the melons at 0, 1, 3, 6, 9 or 15 day post inoculation under the surface of the wash solution, rotating by hand to assure complete coverage and contact of surfaces with solution for 2 min. Washed melons were allowed to dried for 1 h in a biosafety cabinet before microbiological analysis or fresh-cut preparation.

Transfer of *L. monocytogenes* from the Rind to the Flesh During Cutting and Growth on Fresh-Cut Pieces

Inoculated and uninoculated cantaloupes stored at 4°C, with or without prior storage and washing treatments, were cut into four sections using a sterile knife. Each section was further cut and the rinds carefully removed. Approximately 100 g of the interior flesh were placed in a Stomacher® bag along with 200 ml UVM broth (Difco) and pummeled for 30 s in a Stomacher (model 400, Dynatech Laboratories, Alexandria, VA) at medium speed followed by incubation at 35°C for 24 h. A 1 ml aliquot of the UVM broth culture was added to 9 ml of Fraser broth (Difco) and incubated at 35°C for 24 h. The A.O.A.C. approved *Listeria* Rapid Test (Oxoid, Ogdenburg, New York) was used to test for the presence of *L. monocytogenes* in the broth culture before or after addition of selective enrichment. Also, samples were plated (0.1 ml/plate) on PALCAM and modified Oxford (Oxoid) selective agar media.

Growth of *L. monocytogenes* on Fresh-Cut Cantaloupe Tissues

In experiments designed to study growth of *L. monocytogenes* on fresh-cut cubes, melon flesh from uninoculated cantaloupes surface sanitized by dipping in chlorine or hydrogen peroxide solution for 5 min was cut into approximately ~3 cm cubes using a sterilized stainless steel knife. The fresh-cut pieces were inoculated by immersion in a *L. monocytogenes* cocktail (10^6 CFU/ml) for 30 s. The inoculated fresh-cut cubes were placed in sterile Stomacher™ bags

and incubated at 4C, 8C or 20°C for up to 15 days. Populations of *L. monocytogenes* recovered from the inoculated fresh-cut pieces were determined by plating (0.1 ml/plate) on PALCAM. The initial inoculum level on the pieces was 3.5 log CFU/g.

Microbiological Analyses

Whole cantaloupe surfaces receiving washing treatments and those not washed were randomly cut with a sterilized stainless steel cork-borer to produce rind plugs of 22 mm in diameter with a surface area (πr^2) of 3.80 cm^2. The rind (50) plugs were blended (Waring commercial blender, speed set at level 5 for 1 min) with 75 ml of 0.1% peptone water. Serial dilutions were prepared in 0.1% peptone water and aliquots (0.1 ml) were plated on different agar media. Plate Count Agar (PCA, Difco, Detroit, MI) incubated at 30°C for 3 days was used for enumeration of mesophilic aerobes. Lactic acid bacteria were enumerated with de Man, Rogosa and Sharp agar (MRS, Oxoid, Hampshire, UK) with incubation at 30°C for 2 days. Yeast and mold were enumerated using Czapek Malt Agar (CMA, Sigma, St. Louis MO). Enterobacteriaceae were determined using a pour plate method on Violet Red Bile Glucose Agar (VRBGA, Oxoid) with an overlay of the same agar after solidification. For *L. monocytogenes*, *Listeria* identification agar (PALCAM, Sigma, MO) containing *Listeria* selective supplement (L-4660, Sigma) was used with incubation at 37°C for 48 h (17). All plating was done in duplicate. In addition, pure cultures of *L. monocytogenes* were surface plated onto PALCAM agar to serve as references for identification. Representative presumptive colonies of *L. monocytogenes* were subjected to analysis by use of API *Listeria* test kits (bioMeriux Marcy l'Etiole, France) for confirmation.

Statistical Analyses

All experiments were done in triplicate with duplicate samples analyzed at each sampling time. Data were subjected to the Statistical Analysis System (SAS; SAS Institute, Cary, NC) for analysis of variance (ANOVA) and the Bonferroni LSD method to determine if there were significant differences ($p<0.05$) between mean values of number of cells recovered after each treatment.

RESULTS AND DISCUSSION

Effect of EtOH Treatment on Native Microflora on Cantaloupe Rind

The population of total mesophilic aerobes on cantaloupe rinds ranged from 5.84 to 6.48 log_{10} CFU/cm^2 and the yeast and mold population ranged from 1.89 to 2.09 log_{10} CFU/cm^2. Treating whole cantaloupe surfaces with 70% EtOH for 1 min reduced the population of aerobic mesophiles by approximately 1 log CFU/cm^2 and the numbers were significantly ($p<0.05$) lower than the untreated melons. Population of yeast and mold reduced by the EtOH treatment was approximately 0.6 log CFU/cm^2 and was not significantly different ($p>0.05$) from the control. Washing with water did not cause a significant reduction ($p>0.05$) of the native microflora (< 0.5 log CFU/cm^2).

Attachment and Survival of *L. monocytogenes* on Cantaloupe Rind

Listeria monocytogenes was not isolated from the surface of the whole cantaloupe purchased from the supermarket prior to inoculation. Treating cantaloupe surfaces with 70% EtOH to reduce the native microflora enhanced the attachment of the pathogen in contrast to the control or those washed with water (Figure. 1). With an inoculum density of 10^8 CFU/ml, 4.1 log CFU/cm^2 of *L. monocytogenes* were recovered from the cantaloupe rinds treated with 70% EtOH while 3.5 log CFU/cm^2 were recovered from the controlled and water-washed melons. For all melons, the populations of aerobic mesophiles and *L. monocytogenes* on the surface of cantaloupe rind both declined while the yeast and mold population increased during storage at 20°C. The population of native mesophilic aerobes declined by 0.5 to 1.0 log under all four experimental conditions. Numbers of *L. monocytogenes* surviving on the cantaloupe surfaces during storage were not significantly different ($p>0.05$) between EtOH treated and untreated melons at either storage temperature. An approximate 1.5 to 2.0 log reduction in the population of *L. monocytogenes* was observed on the melon surface treated with 70% EtOH and a 1.0 to 1.5 log reduction on those not treated at the end of 15 days storage at 20°C. Yeast and mold populations gradually increased both on treated or untreated melons during storage at 20°C for up to 15 days with an overall increase of approximately 2.5 log 20°C. Francis and O'Beirne (*13*) reported growth of *L. innocua* on lettuce that had been dipped in chlorine and had a reduced population of native microflora. This was not observed on cantaloupe rind when the initial population of native microflora was reduced by treatment with EtOH. The slight increase in the population of yeast and mold on melon surfaces could have resulted from a slight amount of moisture retained after inoculation even after the 1 h drying period. The increased yeast and mold population on the melon surface may have out competed *L. monocytogenes* for colonizable space and available nutrients thus resulting in the observed decline of *L. monocytogenes* at both storage temperatures.

It is possible that the native microflora of cantaloupe especially the yeast and mold population may have interfered with survival or growth of *L. monocytogenes*. Previous studies have reported antagonism by native microflora against *L. innocua* on shredded lettuce (*20*). Attachment of *L. monocytogenes* to cantaloupe rind was slightly increased after reducing the population of native microflora by treatment with ethanol. However, it is not clear if this is due to the presence of a finite number of microbial binding sites that are increased in availability after ethanol treatment due to removal of native microflora and/or some chemical/physical changes to the melon surface that are imparted by the ethanol treatment. All EtOH treated whole cantaloupe surfaces appeared dry and flaky after 1 day storage at 20°C or 3 days at 4°C.

Effect of Washing Treatments

The effect of washing treatments on total mesophilic aerobes, yeast and mold and *L. monocytogenes* on the surfaces of whole cantaloupe stored at 4°C for 24 h is shown in Figure 2. Washing with water did not cause a significant ($p<0.05$) reduction of native microflora or *L. monocytogenes*. However, chlorine and hydrogen peroxide treatments did cause a significant ($p<0.05$) reduction of native microflora and *L. monocytogenes*. Chlorine or hydrogen peroxide treatments both reduced the native population of mesophilic aerobes by approximately 3 $\log_{10}$ CFU/cm^2 and yeast and mold by 1.5 log for EtOH treated and untreated melons. Both treatments also were equally effective in reducing *L. monocytogenes*. The surviving population of *L. monocytogenes* on all cantaloupe surfaces was below the detection limit (2 CFU/cm^2) following the chlorine or hydrogen peroxide washes (a 3 to 4 log reduction). The effect of the

various washes against the microbes after more prolonged storage of inoculated melons as seen in figures 3 and 4 were similar to figure 2. As noted previously washing with water did not cause significant ($p<0.05$) reduction of native microflora or of *L. monocytogenes*, but chlorine and hydrogen peroxide treatments did. Differences noted after 5 and 15 days of storage (Figure 3 and 4, respectively) were; 1) at days 5 and 15 the chlorine and the hydrogen peroxide washes did not completely eliminate yeast and mold and 2) *Listeria monocytogenes* was more susceptible to removal by washing with water especially at day 15. Populations of *L. monocytogenes* did not become more difficult to reduce with time of storage after inoculation. Previously, we reported the inability of chlorine or hydrogen peroxide treatments to significantly reduce attached *Salmonella* Stanley (*33*) or *E. coli* (*32*) on cantaloupe rind after 3 days or more of storage at 4°C post inoculation.

Chlorination of wash water to prevent microbial contamination in produce processing lines is commonly employed (*5,36*), but the formation of potentially carcinogenic chlorinated organic compounds is a concern (*34*). The use of alternative sanitizers such as hydrogen peroxide might be desirable to decontaminate cantaloupe surfaces before fresh-cut preparation (*6*). Efficacy of hydrogen peroxide in preservation of fresh-cut melon (*27*) and vegetables (*19*) and for washing of fresh mushrooms (*26*) has been reported. The results of this study suggest that a hydrogen peroxide treatment to decontaminate cantaloupe surfaces before fresh-cut preparation was comparable to that of a high level of chlorine.

Transfer of *L. monocytogenes* to Fresh-Cut Pieces During Cutting

Results of experiments on the transfer of *L. monocytogenes* from the rind to the melon flesh during fresh-cut preparation are shown in Table 1. Two of three samples of fresh-cut pieces prepared from whole cantaloupe inoculated to give a *L. monocytogenes* population of 2.16 log CFU/cm^2 on the rind plugs were negative for the pathogen even after enrichment. When the population of *L. monocytogenes* on the rind plugs was 3.26 log CFU/cm^2 or above, the pathogen was recovered from all samples of the fresh-cut pieces without enrichment. The results of this study suggest that transfer of the pathogen from the rind to fresh-cut pieces may occur on a fairly consistent basis if the number of *L. monocytogenes* on the rind is $\geq$ 2 log CFU/cm^2. Previously, we reported recovery of *Salmonella* Stanley from fresh-cut pieces prepared from cantaloupe inoculated at 10^3 CFU/cm^2 of rind or above (*33*). Gayler et al. (*14*) showed that the interior tissue of watermelon could be contaminated if *Salmonella* was present either on the rind of the watermelon or on the knife used for slicing. They failed to report the initial inoculum size or the final population attained on the watermelon flesh. Similarly, transfer of *Salmonella* from the surface of tomatoes to the interior during cutting has been reported (*21*). Their data suggested that the rate of bacterial transfer is dependent on inoculum size on the stem scar.

Table 2 shows recovery of *L. monocytogenes* from fresh-cut pieces prepared from washed or unwashed cantaloupes stored at 4°C for up to 15 days post inoculation. *Listeria monocytogenes* was recovered from fresh-cut pieces prepared from all control or water-washed cantaloupes up to 24 h post inoculation. At 5 days post inoculation, fresh-cut pieces prepared from one out of three melons for the control or the water-washed treatments were positive after enrichment for the pathogen. However, fresh-cut pieces prepared from the control or water washed melons 10 or 15 days post inoculations were negative for the pathogen. All samples of fresh-cut pieces prepared from the chlorine or hydrogen peroxide washed melons were negative for the pathogen after enrichment irrespective of time of washing and fresh-cut preparation following days of post

inoculation. The data suggest that sanitation of cantaloupe surfaces used for fresh-cut preparation can help to ensure microbiological safety of fresh-cut melons.

Growth and Survival of *L. monocytogenes* on Fresh-Cut Pieces

Minimally processed fresh fruits and vegetables often provide a good substrate for microbial growth (*22,24*). Readily available nutrients may allow proliferation of human pathogenic organisms like *L. monocytogenes* on fresh-cut melon. Figures 5 shows changes in population of aerobic mesophiles (APC), yeast and mold and *L. monocytogenes* on the surface of inoculated cantaloupe fresh-cut pieces prepared from EtOH, chlorine treated or untreated whole cantaloupe during storage at 8°C for 15 days. In our study *L. monocytogenes* directly inoculated on fresh-cut pieces of cantaloupe survived, but did not grow, during storage at 4°C for up to 15 days (data not shown). *L. monocytogenes* in control fresh-cut pieces survived up to day 8 and was below detection thereafter. The pathogen survived and did not grow in fresh-cut pieces from EtOH and chlorine treated whole melon during storage at 8°C for 15 days. The population for native mesophilic aerobes and yeast and mold increased in all samples irrespective of treatments before fresh-cut preparation. Earlier studies have shown that *L. monocytogenes* is capable of growing on fresh-cut apple slices (*9*) and several vegetables (*1,3,7,20,29,30,31*) stored under controlled or uncontrolled atmosphere conditions. Our study indicates that the pathogen has the ability to grow on fresh-cut cantaloupe at abusive temperatures if the population of *Listeria* is higher than that of native microflora.

In conclusion, *L. monocytogenes* did not grow on inoculated cantaloupe rinds under all conditions tested. Pathogen populations gradually declined, but survivors were detected after 15 days of storage at 4 or 20°C. Sanitizing whole cantaloupe surfaces with chlorine (1000 ppm) and hydrogen peroxide (5%) appears effective in reducing *L. monocytogenes*, mesophilic aerobes and yeast and mold resulting in 2.0 to 3.5 log reductions of *L. monocytogenes* under the various experimental conditions. The effectiveness in reducing the population of *L. monocytogenes* is not dependent on the length of storage after inoculation and before treatment. Treatment of cantaloupe surfaces with 70% EtOH was not an effective means of sanitizing cantaloupe surfaces. Transfer of *L. monocytogenes* from the inoculated rind to the interior flesh during preparation of fresh-cut pieces was demonstrated. Sanitizing whole cantaloupe surfaces with chlorine and hydrogen peroxide at the level used in the study can greatly reduce or eliminate this transfer. Once present on fresh-cut pieces *L. monocytogenes* appears capable of surviving long term storage (5 days) at 4°C.

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